

What is claimed is:
CLAIMS

10/18/01

- Sub C1 > 1. A peptide comprising at least 9 contiguous amino acids of SEQ.ID.NO.1.
 2. A peptide comprising the amino acid sequence of SEQ.ID.NO.3 or a functional fragment thereof.

- Sub A2 > 3. A peptide according to claim 1 or 2 exhibiting trypanolytic activity preferably in combination with cytolytic and/or glucan binding and/or LPS binding and/or opsonizing activity.
 4. An antibody specifically recognizing the peptide of any of the preceding claims or a fragment or epitope thereof.

- Sub C1 > 5. A DNA sequence encoding an Eisenia foetida protein or polypeptide or encoding an immunologically active and/or functional fragment thereof selected from the group consisting of

- (a) DNA sequences comprising a nucleotide sequence encoding a protein or peptide comprising the amino acid sequence as given in SEQ ID NO. 1 or 3;
 (b) DNA sequences comprising a nucleotide sequence as given in SEQ ID NO: 2;
 (c) DNA sequences hybridizing with the complementary strand of a DNA sequence as defined in (a) or (b) and encoding an amino acid sequence which is at least 80% identical to the amino acid sequence encoded by the DNA sequence of (a) or (b);
 (d) DNA sequences the nucleotide sequence of which is degenerated as a result of the genetic code to a nucleotide sequence of a DNA sequence as defined in any one of (a) to (c); and
 (e) DNA sequences encoding a fragment of a protein encoded by a DNA sequence of any one of (a) to (d).

- Sub A3 > 6. A recombinant expression vector comprising a DNA sequence according to claim 5.

- Sub C1 > 7. A host cell transformed or transfected with an expression vector according to claim 6.

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8. The host cell of claim 7 wherein the host cell is selected from the group consisting of *E. Coli*, *Bacillus sp.*, *Streptomyces sp.*, yeast, fungi, insect cells, plant cells or mammalian cells.

9. The host cell of claim 8, wherein the host cell is *E. Coli*.

10. A method for the production of an *Eisenia foetida* polypeptide or an immunologically active or functional fragment thereof comprising culturing a host cell of claim 7, 8 or 9 under conditions allowing the expression of said polypeptide and recovering the produced polypeptide from the culture.

Sub A 6)

12. Use of a peptide according to claim 1, 2 or 3 for the preparation of a medicament to treat trypanosomal infection, bacterial infection or cancer.

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Table 1 : aminoacid sequence of CCF-1 and TNF/TIP peptides

Peptide	Amino acid sequence
CCF-1.1	N-terminus : NH ₂ -FTDWDQYHIVWQDEFDYFDGAKWQHEVTAT-COOH
CCF-1.2	(R,K) ↓ NH ₂ -VYK-COOH
CCF-1.4	(R,K) ↓ NH ₂ -NTGGEFLGIQK-COOH
CCF-1.5	(R,K) ↓ NH ₂ -MGSTMHWGPGWDDNER-COOH
CCF-1.8	(R,K) ↓ NH ₂ -YWLTSLPK-COOH
CCF-1.10 (CCF-1/TIP)	(R,K) ↓ NH ₂ -SGEIDIETIGNR-COOH
TNF/TIP	TPEGAEA

Table 2 : trypanolytic activity of CF and CCF-1.

CF tested ^a	Neutralizing antibody ^d (12C9)	% Trypanolysis	% Inhibition
1. Total CF ^b	-	97	
	+	10	90
2. CF flow through ^b (irrelevant IgG column)	-	94	
	+	7	93
3. CF flow through ^b (12C9 column)	-	30	
	+	2	94
4. Eluate (CCF-1) ^c (12C9 column)	-	42	
	+	0	100

a : CF and CF subfractions were purified by immunoaffinity on irrelevant IgG or 12C9 column and tested for trypanolytic activity in the trypanolysis assay (% trypanolysis was recorded after 2 hrs).

b : Concentration used = 1 mg/ml.

c : Concentration used = 4 µg/ml.

d : 12C9 antibody was added at a concentration of 10 µg/ml.

Table 3 : inhibition of the trypanolytic activity (*T. brucei*) of CCF-1 and TNF- α by antibodies and carbohydrates

Inhibitor ^a	CCF-1 mediated trypanolysis ^b		TNF- α mediated trypanolysis ^c	
	% Lysis	% Inhibition	% Lysis	% Inhibition
None	42	-	41	-
N,N-diacetylchitobiose	3	73	0	100
Cellobiose	49	0	41	0
Polyclonal anti-TNF/TIP	0	100	0	100
Polyclonal IgG control	46	0	43	0
Monoclonal anti-TNF/TIP	0	100	0	100
Monoclonal IgG control	49	0	41	0
Monoclonal anti-CCF-1(12C9)	0	100	1	98
Monoclonal anti-TNF(1F31F3)	44	0	41	0

a : Inhibitors were added at a final concentration of 10 μ g/ml.

b : CCF-1 was added in the trypanolysis assay at a final concentration of 4 μ g/ml.

c : TNF- α was added in the trypanolysis assay at a final concentration of 1.000 U/ml.

Inhibitor ^a	CF-1 mediated trypanolysis ^b	
	% Lysis	% Inhibition
None	62	-
N,N'-diacetylchitobiose	19	70
Cellobiose	67	0
Monoclonal anti-CCF-1(12C9)	30	52
Monoclonal IgG control	67	0

b : CF was added in the trypanolysis assay at a final dilution of 1 : 4.000.

Table 5 : inhibition of the cytolytic activity of CCF-1 (L929) by antibodies and carbohydrates

Inhibitor ^a	CCF-1 mediated cytotoxicity ^b	
	% Lysis	% Inhibition
<u>Experiment 1</u>		
None	72	-
N,N'-diacetylchitobiose	0	100
Monoclonal anti-CCF-1(12C9)	0	100
Monoclonal anti-TNF/TIP	0	100
<u>Experiment 2</u>		
None	66	-
Monoclonal anti-CCF-1(12C9)	14	79
Monoclonal anti-CCF-1(7F1)	0	100
Monoclonal anti-CCF-1(6H1)	0	100

a : Inhibitors were added at a final concentration of 10 µg/ml

b : CCF-1 was added in the L929 cytotoxicity assay at a final concentration of 4 µg/ml

	Parasites x 10 ⁶ /ml	
Day pi	Control mAb-treated	anti-CCF-1 treated
3	104	135
4	129	194
5	64	84
6	2	2

Table 7 : parasitaemia in untreated or CCF-1-treated mice (group of 4 mice)

Day pi	Parasites x 10 ⁶ /ml	
	untreated	rCCF-1 treated
3	207	142
4	211	143
5	102	104
6	6	1.2

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Table 8: Production of TNF- α by 2C11-12 activated with CCF-1

$\mu\text{g/ml}$ CCF-1	pg/ml TNF- α
40	5843
20	2483
10	1112
5	370
2.5	60
1.25	17
0.625	Nd

nd: not detectable

Table 9: Production of TNF- α by C3H/J PECs activated with CCF-1

$\mu\text{g/ml}$ CCF-1	pg/ml TNF- α	
	- IFN- γ	+ IFN- γ
40	nd	300
20	nd	130
10	nd	30
5	nd	Nd
2.5	nd	Nd
1.25	nd	Nd
0.625	nd	Nd

nd: not detectable